Volume 289, number 1, 8–10 FEBS 10088 September 1991

ADONIS 0014579391007995

Tissue-specific expression of Na,K-ATPase β-subunit

Does β2 expression correlate with tumorigenesis?

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Received 24 June 1991

We have found a substantial decrease in the level of Na,K-ATPase β2-subunit mRNA in xenografts of human renal, lung hepatocellular carcinomas in nude mice as compared with corresponding normal tissues, as well as in the neuroblastoma cell line as compared with the neuron primary cell culture. The level of β1 mRNA is decreased in kidney and lung tumor cells, but is unchanged in hepatocellular carcinoma. In the neuroblastoma cell line the level of β1 subunit mRNA was found to be higher then in neuron primary cell culture. The level of α1 mRNA in investigated tumors was the same as in normal tissues. These results may give evidence of the involvement of β2-subunit in the process of tumorigenesis as was shown for some other adhesion molecules.

Na,K-ATPase; β-subunit; Adhesion molecule; Tumorigenesis

1. INTRODUCTION

Na,K-ATPase in the plasma membrane of animal cells is responsible for the active transport of Na^+ and K^+ ions against their electrochemical gradients by means of energy created by ATP hydrolysis. The enzyme consists of 2 subunits, α and β. The α-subunit is a catalytic one, the functional role of the β-subunit remains a matter of discussion. It was supposed that the β-subunit serves as an anchor for the insertion into the membrane of the α-subunit [1]. The discovery of the β2-isoform as an adhesion molecule on glia (AMOG) which was found in astrocytes, clearly showed the participation of the β2-subunit in intercellular interactions, that is in specific neuron–glial contacts [2,3].

By using the technique of molecular cloning, several laboratories have discovered gene families related to the α- and β-subunit of Na,K-ATPase [4–6]. We [7,8] and others [9,10] have shown the tissue-specific manner of expression of different genes that encode isoforms of the Na,K-ATPase α-subunit.

Throughout these investigations we studied the level of mRNAs encoding the α- and β-isoforms of Na,K-ATPase in normal cells and tumor cells. We have found a decreased of β2 mRNA in all tumor cells studied.

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2. MATERIALS AND METHODS

All tissues were obtained by autopsy 4–6 h after death from 25–40-year-old donors who died in accidents. Human tumors transplanted into nude mice were obtained from Dr E. Revasova. Total cellular RNA was isolated from 1 g of tissue or from 5×10^6–10^7 cells according to the method of Feramisco et al. [11]. The level of α- and β-subunit isoform specific mRNAs was determined by use of PCR-amplification.

PCR was used in a semi-quantitative modification: a cDNA first strand was synthesized using 1 μg of total RNA and a random primer. This cDNA was then used in all subsequent amplification reactions by adding corresponding specific primers. 1/10 of the cDNA was amplified in a Perkin-Elmer-Cetus thermal cycler: 94°C, 30 s, 60°C, 30 s, 72°C, 30 s, 36 cycles. In control experiments we have shown that under these conditions there is a proportional relationship between RNA concentration and the strength of the signal (by taking pictures of agarose gels stained by EtBr in UV-light).

The following primers were used as α1-specific: 5′- AAGAACTG-CCTGGTGAAGAACCTGGAC-3′ and 5′-AGCTGAAGTCTTGT-CAAAAGAGAC-3′; as β1-specific: 5′-ACTGAAATTTCCTTTC-GTCCTAAT-3′ and 5′-ATCACTGGGTAAGTCTCCA-3′; and as β2-specific: 5′-CTTGATGTCATTGTCAATGTCAGT-3′ and 5′-TCGATGTTGCCGTTGGCGGGGAAC-3′. As a control we amplified a fragment of human β-actin gene. The amplification products were analyzed by gel electrophoresis in a 2% agarose gel containing 0.5 mg/ml ethidium bromide. The pictures from the gels were taken in UV-light using Polaroid 665 film and negatives were scanned on the Apple scanner (AMS, England).

3. RESULTS AND DISCUSSION

Figure 1 shows the results of the amplification of Na,K-ATPase α1, β1- and β2-specific mRNA fragments in normal human kidney, lung, liver tissues and corresponding tumor cells (other isoforms of Na,K-

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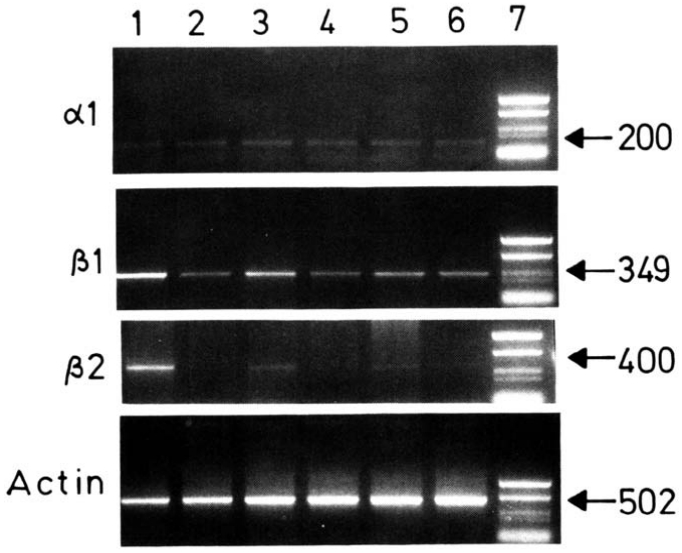

Fig. 1. PCR analysis of Na,K-ATPase mRNAs distribution in normal and tumor human tissues. The length of amplified fragments is indicated to the right. Lanes 1,2,3,4,5,6, PCR from RNA isolated from human kidney normal tissues, renal tumor, lung normal tissue, lung tumor, liver normal tissue and hepatic tumor, respectively. Lane 7, markers pUC19/*Sau*3A.

ATPase α-subunit are not expressed or are expressed in very small amounts in these tissues [12]). Figure 2 illustrates the results of the analysis of expression of the isoforms in the primary culture of neurons compared with the neuroblastoma cells, line IMR32 (neuroblast-like (N) and fibroblast-like (S) [12,13]). As a control the results of the amplification of human β-cytoplasmic actin mRNA are given. These show that all probes contained a roughly equal amount of RNA and also demonstrate the standard conditions of amplification. Figure 3 presents the quantitative estimation of results of Figs 1 and 2.

It follows from Figs 1 and 2 that the level of Na,K-ATPase α1 mRNA does not change significantly when normal and tumor cells are compared. The level of β1 mRNA in renal and lung cell carcinomas was decreased compared with normal tissues, but in hepatocellular tissues it was the same as in normal tissues. The level of β1 in the neuroblastoma cell line was higher than in neurons (Fig. 2). In contrast we observed a decreased level of β2 mRNA in all tumors investigated (Fig. 1 and 2).

The decrease in the level of the β2 mRNA in cancer cells as compared with normal tissues deserves special attention because recent results give evidence that different adhesion molecules play an important role in processes of tumorigenesis [14–16]. Recently it was shown that the β2-subunit of Na,K-ATPase is an adhesion molecule on glia (AMOG) [2,3]. The results of our investigation give evidence that this molecule may play some role in tumor formation. However, the possibility that the effect observed is only an accompanying one cannot be excluded.

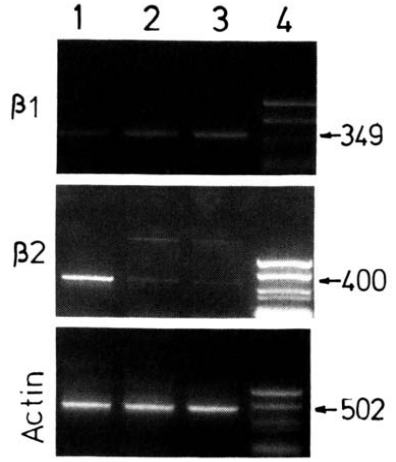

Fig. 2. PCR analysis of Na,K-ATPase β mRNAs distribution in human neurons and neuroblastoma IMR32 cells. Lanes 1,2,3, PCR from RNA of neurons, neuroblastoma cells (N-type), neuroblastoma cells (S-type), respectively. Lane 4, size markers, pUC19/*Sau*3A.

Numerous studies have noted the similarity in amino acid sequences between cell surface glycoproteins which are supposed to be involved in the neoplasia formation. Therefore we have searched in a protein sequence data bank (National Biological Research Foundation Protein Identification Resource (USA) release number 22) for proteins which share homology with β-subunits of Na,K-ATPase. Our preliminary results revealed two fragments homologous with different members of the Ig-superfamily (data not shown). Therefore the possibility that β-subunits and the proteins of this superfamily have a common origin but diverged early in evolution cannot be excluded. The results of the homology search will be published elsewhere.

If AMOG/β2 is involved in the process of transformation the question then arises as to whether it exerts its function as part of Na,K-ATPase or apart from it.

Despite the fact that our results do not enable us to make final conclusions they give some evidence to suggest that the β-subunit of Na,K-ATPase and β2 in par-

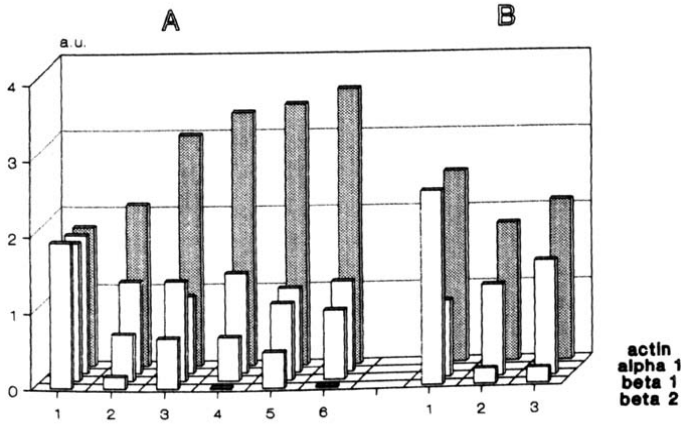

Fig. 3. Histograms quantifying the level of Na,K-ATPase mRNAs in different human cells. (A) Corresponds to the results presented in Fig. 1. (B) Corresponds to the results presented in Fig. 2. a.u. arbitrary units.

ticular may function apart from the α-subunit. In fact, Figs 1 and 2 show that in tumor cells there is no balance in the content of the α and β mRNAs in comparison with normal tissues: while the level of α1 mRNA is relatively constant, both β1 and β2 mRNAs are significantly reduced in renal and lung tumors.

When different types of cells were compared [9], cases were observed where there was no co-regulation, at least at the RNA level, of α- and β-subunits. The authors suppose that if the synthesis of the α- and β-subunits of Na,K-ATPase is coordinately regulated, then the mechanism is dependent upon cell type [9]. Marxer et al. [17] have not found, Na,K-ATPase activity in the brush border membrane of the distal colon although a β-subunit of Na,K-ATPase or β-like protein has been detected. The proposal regarding the possibility of separate functions for β-subunits was also put forward by Good et al. [18] who found a nervous system-specific isoform of β-subunit, β3, expressed during early development of *Xenopus laevis*.

In summary, the results obtained allow the formulation of several questions of general and particular value: (i) is there a causal-consequence relation between β2 expression and tumorigenesis? (ii) what molecular mechanisms provoke a decrease in the level of β2 in cases of tumor formation? and (iii) do β-subunits exert other functions apart from ion transport? One can thus hope that answering these questions will shed light on the mechanism of Na,K-ATPase functioning as well as on the mechanisms of tumorigenesis.

Acknowledgements: We thank Dr E.S. Revasova for generously providing us with samples of human tumors. We also thank Dr Yu. Balabanov for the neuron primary cell culture and Dr N. Vinogradova for neuroblastoma cells cultivation.

REFERENCES

[1] Zamofing, D., Rossier, B.C. and Geering, K. (1988) J. Membr. Biol. 104, 69–79.
[2] Pagliusi, S., Antonicek, H., Gloor, S., Frank, R., Moos, M. and Schachner, M. (1989) J. Neurosci. Res. 22, 113–119.
[3] Gloor, S., Antonicek, H., Sweadner, K.J., Pagliusi, S., Frank, R., Moos, M. and Schachner, M. (1990) J. Cell. Biol. 110, 165–174.
[4] Sverdlov, E.D., Monastyrskaya, G.S., Broude, N.E., Ushkaryov, Yu. A., Allikmets, R.L., Melkov, A.M., Smirnov, Yu. V., Malyshev, I.V., Dulubova, I.E., Petrukhin, K.E., Grishin, A.V., Kijatkin, N.I., Kostina, M.B., Sverdlov, B.E., Modyanov, N.N. and Ovchinnikov, Yu. A. (1987) FEBS Lett. 217, 275–278.
[5] Shull, G.E., Greeb, J. and Lingrel, J.B. (1986) Biochemistry 25, 8125–8132.
[6] Shull, M.M. and Lingrel, J.B. (1987) Proc. Natl. Acad. Sci. USA 84, 4039–4043.
[7] Sverdlov, E.D., Akopyanz, N.S., Petrukhin, K.E., Broude, N.E., Monastyrskaya, G.S. and Modyanov, N.N. (1988) FEBS Lett. 239, 65–68.
[8] Broude, N.E., Modyanov, N.N., Monastyrskaya, G.S. and Sverdlov, E.D. (1989) FEBS Lett. 257, 1–9.
[9] Young, R.M. and Lingrel, J.B. (1987) Biochem. Biophys. Res. Commun. 145, 52–58.
[10] Emanuel, J.R., Garetz, S., Stone, S. and Levenson, R. (1987) Proc. Natl. Acad. Sci. USA 84, 9030–9034.
[11] Feramisco, J.R., Helfman, D.M., Smart, J.E., Burridge, K. and Thomas, G.P. (1982) J. Biol. Chem. 257, 11024–11031.
[12] Akopyanz, N.S., Broude, N.E., Vinogradova, N.G., Balabanov, Yu. A., Monastyrskaya, G.S. and Sverdlov, E.D. (1991) in: The Sodium Pump: Recent Developments, The Rockefeller University Press, in press.
[13] Ciccarone, V., Spengler, B.A., Meyers, M.B., Biedler, J.L. and Ross, R.A. (1990) Cancer Res. 49, 219–225.
[14] Giancotti, F.G. and Ruoslahti (1990) Cell 60, 849–859.
[15] Fearon, E.R., Cho, K.R., Nigro, J.M., Kern, S.E., Simons, J.W., Ruppert, J.M., Hamilton, S.R., Preisinger, A.C., Thomas, G., Kinzler, K.W. and Vogelstein, B. (1990) Science 247, 49–56.
[16] Inghirami, G., Grignani, F., Sternas, L., Lombardi, L., Knowles, D.M. and Dalla-Favera, R. (1990) Science 250, 682–686.
[17] Marxer, A., Stieger, B., Quaroni, A., Kashgarian, M. and Hauri, H.-P. (1989) J. Cell. Biol. 109, 1057–1069.
[18] Good, P.J., Richter, K. and David, I. (1990) Proc. Natl. Acad. Sci. USA 87, 9082–9092.